



# Modeling Severe Fever with Thrombocytopenia Syndrome Virus Infection in Golden Syrian Hamsters: Importance of STAT2 in Preventing Disease and Effective Treatment with Favipiravir

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**ABSTRACT** Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease endemic in parts of Asia. The etiologic agent, SFTS virus (SFTSV; family *Bunyaviridae*, genus *Phlebovirus*) has caused significant morbidity and mortality in China, South Korea, and Japan, with key features of disease being intense fever, thrombocytopenia, and leukopenia. Case fatality rates are estimated to be in the 30% range, and no antivirals or vaccines are approved for use for treatment and prevention of SFTS. There is evidence that in human cells, SFTSV sequesters STAT proteins in replication complexes, thereby inhibiting type I interferon signaling. Here, we demonstrate that hamsters devoid of functional STAT2 are highly susceptible to as few as 10 PFU of SFTSV, with animals generally succumbing within 5 to 6 days after subcutaneous challenge. The disease included marked thrombocytopenia and inflammatory disease characteristic of the condition in humans. Infectious virus titers were present in the blood and most tissues 3 days after virus challenge, and severe inflammatory lesions were found in the spleen and liver samples of SFTSV-infected hamsters. We also show that SFTSV infection in STAT2 knockout (KO) hamsters is responsive to favipiravir treatment, which protected all animals from lethal disease and reduced serum and tissue viral loads by 3 to 6 orders of magnitude. Taken together, our results provide additional insights into the pathogenesis of SFTSV infection and support the use of the newly described STAT2 KO hamster model for evaluation of promising antiviral therapies.

**IMPORTANCE** Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral disease for which there are currently no therapeutic options or available vaccines. The causative agent, SFTS virus (SFTSV), is present in China, South Korea, and Japan, and infections requiring medical attention result in death in as many as 30% of the cases. Here, we describe a novel model of SFTS in hamsters genetically engineered to be deficient in a protein that helps protect humans and animals against viral infections. These hamsters were found to be susceptible to SFTSV and share disease features associated with the disease in humans. Importantly, we also show that SFTSV infection in hamsters can be effectively treated with a broad-spectrum antiviral drug approved for use in Japan. Our findings suggest that the new SFTS model will be an excellent resource to better understand SFTSV infection and disease as well as a valuable tool for evaluating promising antiviral drugs.

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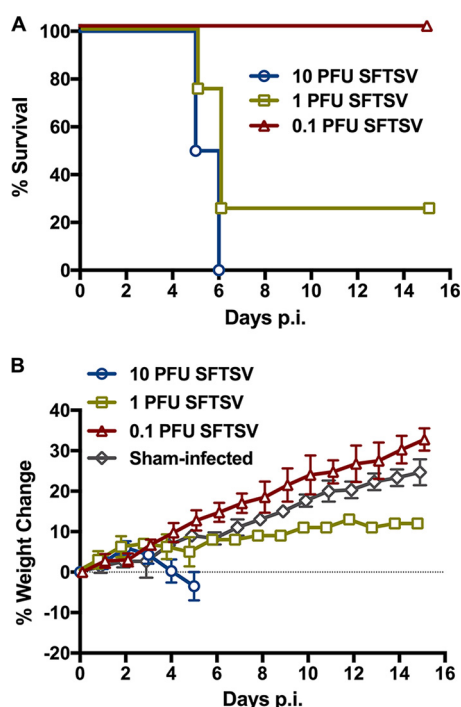
Severe fever with thrombocytopenia syndrome virus (SFTSV) is a tick-borne phlebovirus (family *Bunyaviridae*) that has recently emerged in parts of Asia and causes a hemorrhagic disease syndrome with the cardinal features being thrombocytopenia and leukopenia (1). Clinical presentation typically includes abrupt onset of fever with headache, myalgia, lethargy, and diarrhea (2). Primarily affecting China, South Korea, and Japan, mortality associated with SFTSV infection can be high, with case fatality rate estimates reaching 30%. There is currently no antiviral therapy approved for the treatment of severe fever with thrombocytopenia syndrome, and there are no vaccines available to prevent the disease.

SFTSV is an enveloped virus with a trisegmented single-stranded RNA genome consisting of a large (L) segment, a medium (M) segment, and a small (S) segment (reviewed in reference 2). The L segment encodes the viral RNA-dependent RNA polymerase required for genome replication and transcription. The M segment contains the envelope glycoprotein, which in addition to mediating host cell entry, facilitates virion assembly and maturation. Using an ambisense coding strategy, the S segment encodes the nucleoprotein and a nonstructural protein (NSs). The nucleoprotein is a multifunctional protein that protects the viral genome, is involved in the replication/transcription process, and has a role in virion assembly (3, 4). The NSs protein of SFTSV has been shown to inhibit the type I interferon (IFN) response through sequestration of STAT proteins and other factors in viral replication complexes (5–7). Within the pathogenic phleboviruses, the NSs protein is considered the major virulence factor, playing a central role in the disruption of the innate immune response to infection by targeting the IFN antiviral response pathway (8).

Immunocompetent mice and golden Syrian hamsters have been shown to be refractory to severe infection and disease following SFTSV challenge; however, mice lacking IFN- $\alpha/\beta$  receptors are highly susceptible and generally succumb from an acute disease course (9–11). In the present study, we demonstrate an essential role for STAT2 in the control of SFTSV infection in hamsters and subsequently characterize the pathogenesis and natural history of disease in hamsters deficient in STAT2 signaling. We also show that favipiravir, a broadly active antiviral drug approved for human use in Japan, completely protects STAT2 knockout (KO) hamsters from lethal SFTSV challenge. The new hamster model of SFTS shares features of the human disease, including hemorrhagic disease pathology and thrombocytopenia, and will further support antiviral drug and vaccine development efforts.

## RESULTS

**Susceptibility of STAT2 KO hamsters to SFTSV.** Wild-type Syrian hamsters are refractory to disease following SFTSV challenge (10). To investigate whether STAT2 deficiency would render hamsters susceptible to SFTSV, STAT2 KO hamsters were inoculated subcutaneously (s.c.) with serial dilutions of SFTSV (10, 1, or 0.1 PFU) or sham infected and observed for signs of illness. A dose of 10 PFU resulted in uniform lethality, with all 4 animals succumbing by day 6 postinfection (p.i.) (Fig. 1A). With the 1-PFU SFTSV challenge, only 1 of the 4 animals survived. There was no mortality observed at a challenge dose of 0.1 PFU (Fig. 1A) or in sham-infected animals (data not shown). All the hamsters challenged with 10 PFU started losing weight by day 3 p.i. (Fig. 1B), and the weight loss coincided with the onset of lethargy in several animals. Animals challenged with 1 PFU had slightly lower weight loss than the animals infected with 10 PFU, and the surviving hamster recovered slowly, as reflected by its modest weight gain curve compared to those of the animals that were infected with 0.1 PFU. The 0.1-PFU- and sham-infected animals did not show any signs of illness and steadily gained weight at a higher trajectory throughout the study than the animals in the other groups (Fig. 1B). Based on the survival results, the 50% and 90% lethal doses (LD<sub>50</sub> and LD<sub>90</sub>)



**FIG 1** Titration of SFTSV in STAT2 KO hamsters. Groups of 4 hamsters were infected by s.c. injection with the indicated PFU of virus. (A) Survival; (B) daily percent changes and standard deviations in the mean weights of surviving animals relative to the day of virus challenge.

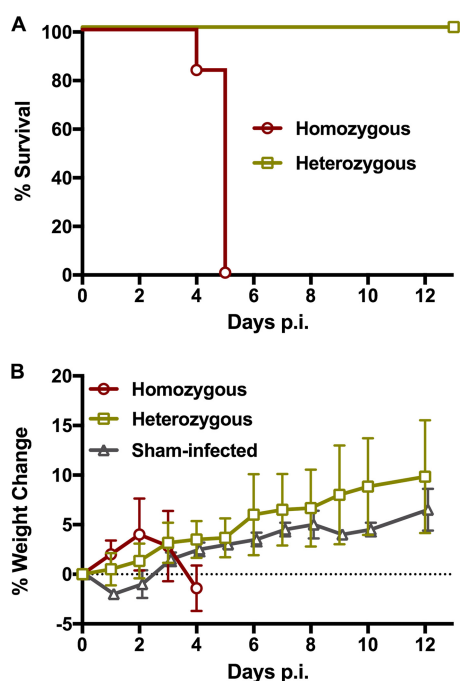
respectively) of the HB29 strain of SFTSV in STAT2 KO hamsters by the s.c. challenge route are approximately 0.5 and 4.6 PFU, respectively.

**Hamsters heterozygous for the STAT2 KO are resistant to lethal SFTSV challenge.** To further investigate the role of STAT2 in the control of SFTSV infection, challenges of STAT2 KO hamsters and heterozygous littermates with a functional STAT2 allele were conducted in parallel. As expected, all of the homozygous STAT2 KO hamsters succumbed to the 100-PFU SFTSV challenge dose by day 5 p.i., with notable weight loss and slightly reduced activity beginning on day 3 (Fig. 2). In contrast, the heterozygous animals showed no signs of illness, suggesting that a single functional copy of the STAT2 gene is sufficient to effectively control SFTSV infection and prevent disease and mortality.

**Pathogenesis of SFTSV in homozygous STAT2 KO hamsters.** To gain insights into the pathogenesis of SFTSV infection, a natural history of disease study was designed to investigate several virologic, clinical, and laboratory parameters during the acute infection in Syrian STAT2 KO hamsters. A challenge dose of 50 PFU of SFTSV was chosen to ensure uniform lethality at 100 LD<sub>50</sub>. SFTSV-infected animals began losing weight on day 3 p.i. (Fig. 3A). By day 4, the remaining infected hamsters had various degrees of lethargy. On the morning of day 5, 1 of the 3 animals was found in a moribund state and the other 2 hamsters had expired.

Serum and tissues were analyzed for the presence of infectious virus during the course of the SFTSV infection. As shown in Fig. 3B, viremia and tissue viral titers were not detectable until day 3 p.i. and were substantial in all tissues, with the exception of the small intestine, by day 4. An amount of  $>10^7$  50% cell culture infectious dose (CCID<sub>50</sub>)/ml serum or gram of tissue (including the small intestine) was found in the moribund animal sacrificed on day 5 p.i. (Fig. 3B).

Hematologic analysis showed that erythrocyte parameters were largely unaffected by the SFTSV infection with the exception of percent red blood cell (RBC) distribution width (RDW%), which was significantly increased 4 days p.i. (Table 1). In contrast, a significant decrease in platelets was observed starting on day 3 p.i., with the day 5

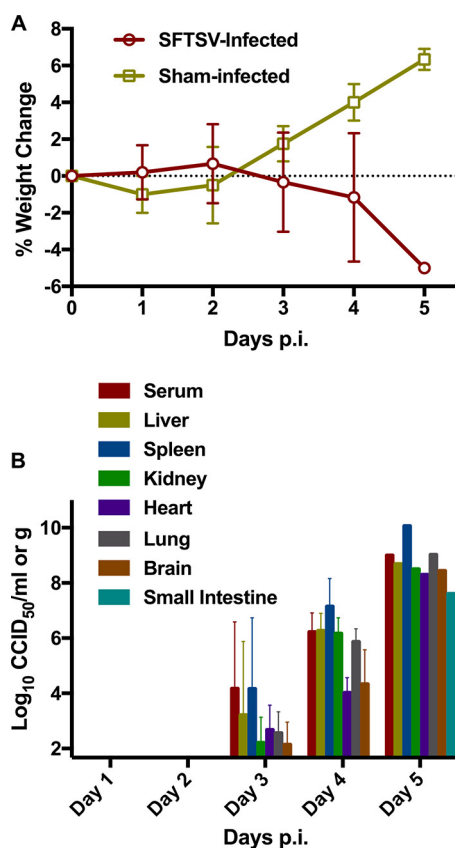


**FIG 2** Lethality of SFTSV infection in *STAT2* KO homozygous and heterozygous hamsters. Animals homozygous ( $n = 6$ ) and heterozygous ( $n = 6$ ) for the *STAT2* KO were infected s.c. with 100 PFU of SFTSV. (A) Survival; (B) daily percent changes and standard deviations in the mean weights of surviving animals relative to the day of virus challenge. Sham-infected controls ( $n = 2$ ) consisted of 1 homozygous animal and 1 heterozygous animal.

hamster having critically low levels (Fig. 4A). Notably, similar amounts of platelet clumps were observed in blood smears across all groups, including the sham-infected control animals, so the reported values are likely an underestimate of the total platelet concentration. To assess whether the thrombocytopenia was due to reduced production of platelets, bone marrow smears were also examined and determined to have no deficits in megakaryocytes (not shown). White blood cell (WBC) counts were estimated by visual analysis of blood smears prepared immediately after blood collection in the biosafety level 3 (BSL-3) laboratory. WBC counts did not differ significantly from those of the sham-infected controls (Table 1). A shift toward increased percentages of neutrophils and decreasing percentages lymphocytes was observed as the SFTSV infection progressed in the hamsters (Fig. 4B and C). This inversion of neutrophil to lymphocyte numbers is indicative of a strong inflammatory response (12). Also consistent with inflammation, immature (band) neutrophils were elevated in 2 of 3 animals on day 4 and in the moribund animal on day 5 (Fig. 4D).

The blood smears were assessed for toxic changes in neutrophils to gain further insights into the inflammatory response during the acute SFTSV infection. Toxic changes are cytoplasmic details (basophilia, vacuolation, Dohle bodies, and granulation) that occur while neutrophils are formed in the bone marrow under a significant cytokine influence (13). The toxic changes were graded on a scale of 0 to 3 with 0 being no toxic changes and 3 being marked toxic changes. As shown in Fig. 4E, toxic changes indicative of a vigorous systemic inflammatory response became pronounced in one of the day 3 group hamsters and all animals by day 4 p.i.

Serum obtained from each animal at the time of sacrifice was also used for a comprehensive analysis of serum biochemistry parameters (Table 2). Most notably, alkaline phosphatase (ALP) and cholesterol (CHOL) levels began to increase by day 3 p.i. and were found to be significantly elevated in the animals sacrificed on day 4. The moribund day 5 animal had dramatically higher concentrations of ALP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT),



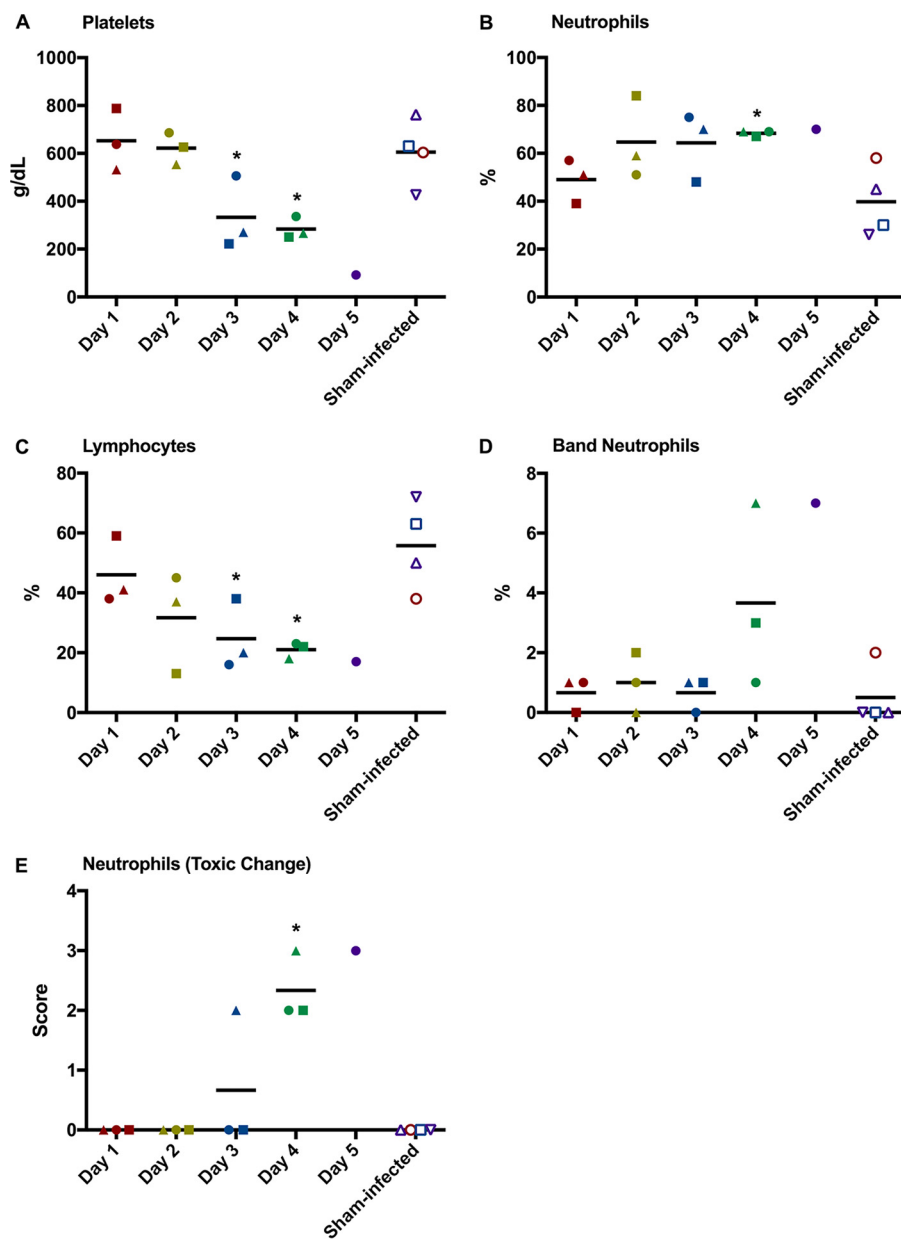
**FIG 3** Temporal analysis of weight change and viral burden during the course of SFTSV infection in *STAT2* KO hamsters. Animals ( $n = 3/\text{day}$ ) were infected s.c. with 50 PFU of SFTSV and sacrificed on the indicated day for analysis of viral titers. (A) Daily percent changes and standard deviations in the mean weights of surviving animals relative to the day of virus challenge; (B) serum and tissue viral titers. Two animals in the day 5 group succumbed to the infection prior to time of sacrifice; therefore, data for only one animal are shown for day 5.

and total bilirubin (TBIL) than the sham-infected controls. There was also a trend toward decreasing concentrations of glucose (GLU) and albumin (ALB) starting 3 to 4 days p.i. with a marked reduction in the day 5 hamster. The day 5 animal and one of the day 4 animals had markedly elevated BUN concentrations.

**TABLE 1** Hematology analysis during the course of the SFTSV infection in *STAT2* KO Syrian golden hamsters<sup>a</sup>

Hematology parameter (unit)	Group mean value $\pm$ SD for infected hamsters on:					Normal value $\pm$ SD
	Day 1	Day 2	Day 3	Day 4	Day 5	
RBC ( $10^6/\mu\text{l}$ )	8.4 $\pm$ 0.2	8.2 $\pm$ 0.2	8.1 $\pm$ 0.6	8.0 $\pm$ 1.3	6.9	9.0 $\pm$ 0.6
HGB (g/dl)	18.7 $\pm$ 0.9	18.4 $\pm$ 0.6	18.2 $\pm$ 1.2	17.2 $\pm$ 2.5	13.5	19.4 $\pm$ 1.2
HCT (%)	55.1 $\pm$ 2.4	51.3 $\pm$ 0.5	52.1 $\pm$ 2.8	48.2 $\pm$ 6.9	42.6	55.7 $\pm$ 2.3
MCV (fl)	<b>65.7 <math>\pm</math> 1.7*</b>	62.4 $\pm$ 1.0	64.0 $\pm$ 1.4	60.0 $\pm$ 1.2	62.1	62.2 $\pm$ 1.6
MCHC (g/dl)	34.0 $\pm$ 0.4	35.8 $\pm$ 0.7	35.0 $\pm$ 0.6	35.7 $\pm$ 0.1	31.6	34.9 $\pm$ 1.0
RDW (%)	18.7 $\pm$ 0.3	18.5 $\pm$ 0.2	19.1 $\pm$ 0.4	<b>19.8 <math>\pm</math> 0.5*</b>	21.7	18.8 $\pm$ 0.4
PLT ( $10^3/\mu\text{l}$ )	652.7 $\pm$ 128.6	622.0 $\pm$ 66.1	<b>332.7 <math>\pm</math> 152.0*</b>	<b>284.0 <math>\pm</math> 45.7*</b>	92.0	605.5 $\pm$ 138.2
MPV (fl)	6.9 $\pm$ 0.1	7.2 $\pm$ 0.2	7.4 $\pm$ 1.6	7.6 $\pm$ 1.3	6.6	6.6 $\pm$ 0.5
WBC ( $10^3/\mu\text{l}$ )	4.3 $\pm$ 1.3	5.9 $\pm$ 3.9	8.3 $\pm$ 7.4	4.8 $\pm$ 2.4	3.2	5.3 $\pm$ 1.2
% Bands	0.7 $\pm$ 0.6	1.0 $\pm$ 1.0	0.7 $\pm$ 0.6	3.7 $\pm$ 3.1	7.0	0.5 $\pm$ 1.0
% Neut	49.0 $\pm$ 9.2	64.7 $\pm$ 17.2	64.3 $\pm$ 14.4	<b>68.3 <math>\pm</math> 1.2*</b>	70.0	39.8 $\pm$ 14.7
% Lymph	46.0 $\pm$ 11.4	31.7 $\pm$ 16.7	<b>24.7 <math>\pm</math> 11.7*</b>	<b>21.0 <math>\pm</math> 2.6*</b>	17.0	55.8 $\pm$ 14.9
% Mono	2.0 $\pm$ 1.0	1.3 $\pm$ 0.6	<b>10.0 <math>\pm</math> 2.6**</b>	5.7 $\pm$ 6.0	6.0	3.0 $\pm$ 2.6
% Eos	2.3 $\pm$ 1.5	1.3 $\pm$ 1.5	0.3 $\pm$ 0.6	1.3 $\pm$ 0.6	0.0	1.8 $\pm$ 1.0

<sup>a</sup>Three animals were sacrificed daily on days 1 to 4 p.i. ( $n = 3$ ). Two animals in the day 5 group succumbed to the infection prior to time of sacrifice; therefore, data for only one animal are shown for day 5. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  compared to sham-infected controls ( $n = 4$ ). Statistically significant differences are in boldface.



**FIG 4** Alteration of hematologic parameters during the course of SFTSV infection in *STAT2* KO hamsters. Groups of hamsters were challenged as described for Fig. 3. Changes in platelets (A), percent neutrophils (B), percent lymphocytes (C), and percent band neutrophils (D) and neutrophil toxic change scores (E) are shown. Unique symbols in each treatment group represent values for the same animal across all parameters. Data for 4 sham-infected, healthy control animals are included for comparison. \*,  $P < 0.05$  compared to sham-infected controls.

Histologically, lesions were observed in the liver and spleen, with other tissues not having microscopic changes (Fig. 5). The liver lesions consisted of multifocal periportal and random, acute, neutrophilic hepatitis (Fig. 5A). Splenic lesions consisted of multifocal acute neutrophilic splenitis focused mostly on the white pulp with lymphoid necrosis (Fig. 5B). Liver lesions were initially observed in hamsters starting 3 days p.i., while splenic lesions were seen as early as day 2 in two animals (Fig. 5E).

**Favipiravir provides complete protection from lethal SFTSV infection in hamsters.** Having characterized SFTSV infection in *STAT2* KO hamsters, we designed an experiment to evaluate two broad-spectrum antiviral drugs in the newly developed disease model. High and intermediate doses of favipiravir (300 mg/kg of body weight/

**TABLE 2** Blood chemistry parameters during the course of the SFTSV infection in *STAT2* KO Syrian golden hamsters<sup>a</sup>

Blood chemistry parameter (unit)	Group mean value $\pm$ SD for infected hamsters on:					Normal mean value $\pm$ SD
	Day 1	Day 2	Day 3	Day 4	Day 5	
ALP (U/liter)	146.3 $\pm$ 9.2	137.7 $\pm$ 9.3	199.3 $\pm$ 37.6	<b>399.7 <math>\pm</math> 50.6***</b>	>993.0	140.8 $\pm$ 15.9
ALT (U/liter)	95.7 $\pm$ 22.6	108.3 $\pm$ 19.3	85.0 $\pm$ 44.0	75.3 $\pm$ 47.9	>1,000	137.0 $\pm$ 85.0
AST (U/liter)	81.3 $\pm$ 3.1	76.7 $\pm$ 8.5	115.7 $\pm$ 49.7	84.3 $\pm$ 26.0	>1,000	126.0 $\pm$ 55.5
BUN (mg/dl)	23.0 $\pm$ 5.8	20.9 $\pm$ 4.4	14.2 $\pm$ 2.4	30.4 $\pm$ 16.5	91.5	20.8 $\pm$ 2.2
CREA (mg/dl)	$\leq$ 0.2	$\leq$ 0.2	$\leq$ 0.2	0.3 $\pm$ 0.1	0.6	$\leq$ 0.2
GLU (mg/dl)	231.7 $\pm$ 59.0	257.3 $\pm$ 27.2	204.0 $\pm$ 78.9	131.3 $\pm$ 52.0	19.0	268.0 $\pm$ 80.0
TP (mg/dl)	6.2 $\pm$ 0.2	6.3 $\pm$ 0.3	6.3 $\pm$ 0.6	6.2 $\pm$ 0.6	4.6	5.8 $\pm$ 0.2
TBIL (mg/dl)	0.2	0.1	0.1	0.1	3.3	0.2 $\pm$ 0.1
ALB (g/dl)	3.1 $\pm$ 0.1	3.2 $\pm$ 0.2	2.9 $\pm$ 0.3	2.6 $\pm$ 0.1	2.0	2.9 $\pm$ 0.2
PHOS (mg/dl)	9.8 $\pm$ 0.8	8.8 $\pm$ 0.3	9.1 $\pm$ 1.3	10.4 $\pm$ 1.1	45.0	10.6 $\pm$ 1.2
Ca (mg/dl)	15.2 $\pm$ 0.5	15.3 $\pm$ 0.3	14.2 $\pm$ 1.1	13.5 $\pm$ 0.4	10.1	14.5 $\pm$ 0.4
CHOL (mg/dl)	164.3 $\pm$ 2.3	163.0 $\pm$ 25.1	227.7 $\pm$ 42.4	<b>269.0 <math>\pm</math> 69.9*</b>	217.0	168.8 $\pm$ 34.9
GGT (U/liter)	10.3 $\pm$ 0.6	<10.0	11.0 $\pm$ 1.0	11.3 $\pm$ 2.3	19.0	10.3 $\pm$ 0.5
LIP (U/liter)	90.3 $\pm$ 23.5	63.3 $\pm$ 49.7	92.7 $\pm$ 25.8	232.0 $\pm$ 186.2	105.0	115.3 $\pm$ 38.9
AMY (U/liter)	>2,500	>2,500	2,412 $\pm$ 153	>2,500	1,525	>2,500
Mg (mg/dl)	5.3 $\pm$ 0.2	5.0 $\pm$ 0.6	5.5 $\pm$ 0.4	5.4 $\pm$ 0.2	6.1	4.7 $\pm$ 1.0
TRIG (mg/dl)	408.3 $\pm$ 100.4	338.7 $\pm$ 29.7	297.7 $\pm$ 42.2	421.7 $\pm$ 131.4	>500.0	360.0 $\pm$ 117.9
Na <sup>+</sup> (mEq/liter)	143.7 $\pm$ 2.1	141.7 $\pm$ 1.5	139.7 $\pm$ 1.2	142.0 $\pm$ 1.0	140.0	141.0 $\pm$ 1.6
K <sup>+</sup> (mEq/liter)	8.8 $\pm$ 0.2	8.8 $\pm$ 0.5	9.5 $\pm$ 1.5	8.4 $\pm$ 1.3	8.3	10.0 $\pm$ 0.3
Cl <sup>-</sup> (mEq/liter)	94.0 $\pm$ 3.6	93.7 $\pm$ 4.0	93.3 $\pm$ 2.9	96.0 $\pm$ 4.4	93.0	93.3 $\pm$ 4.4

<sup>a</sup>Three animals were sacrificed daily on days 1 to 4 p.i. ( $n = 3$ ). Two animals in the day 5 group succumbed to the infection prior to time of sacrifice; therefore, data for only one animal are shown for day 5. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  compared to sham-infected controls ( $n = 4$ ). Statistically significant differences are in boldface.

day and 150 mg/kg/day) and a single dose of ribavirin (75 mg/kg/day) were tested. As shown in Fig. 6A, treatment with either dose of T-705 provided complete protection against a lethal SFTSV challenge. In contrast, none of the animals receiving ribavirin therapy survived the infection, although there appeared to be a slight delay in time of death. As expected, all the animals in the placebo group succumbed to infection by day 5 p.i.

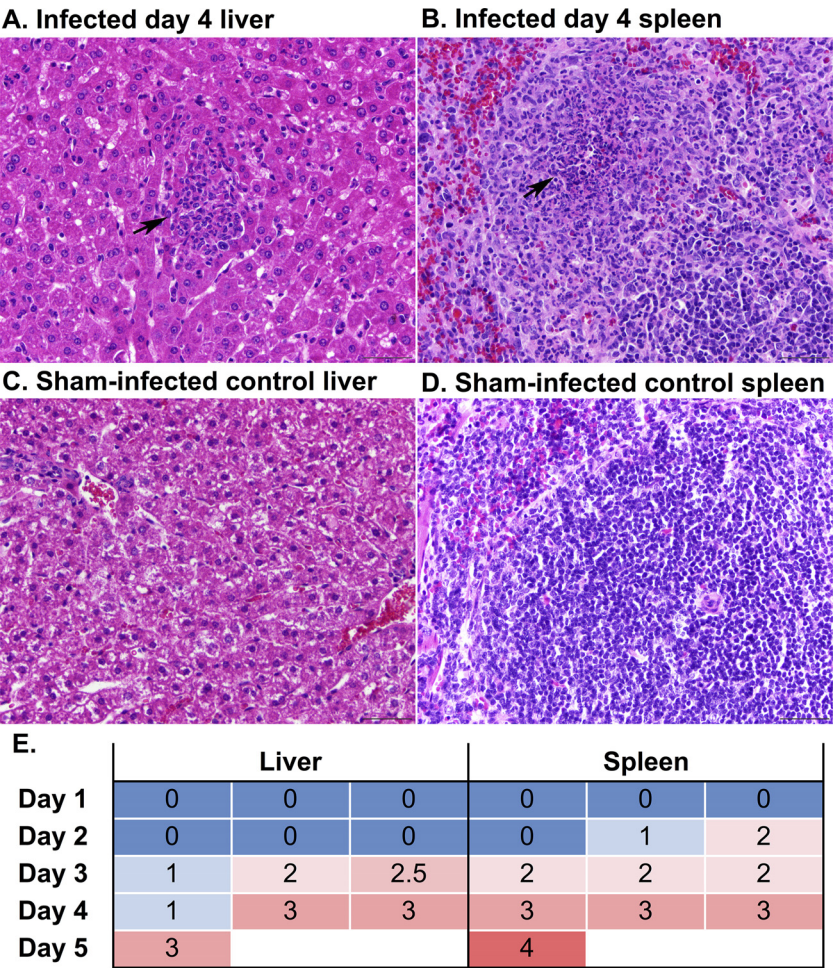
Hamster weights were also tracked during the course of the efficacy study (Fig. 6B). The weight change data are indicative of illness in both of the favipiravir treatment groups and is consistent with the reduced activity that was observed mostly 5 to 10 days p.i. in these treatment groups. A dose-dependent effect was evident as the 300-mg/kg/day favipiravir group lost less weight and began to recover earlier than the animals receiving 150 mg/kg/day favipiravir. The rapid weight loss in the ribavirin-treated animals is consistent with the survival data.

The effect of treatments on reducing day 4 viral titers was evaluated in subsets of hamsters infected and treated in parallel to those observed for mortality (Fig. 6C). In a dose-responsive manner, animals treated with favipiravir showed significant reductions in viremia levels in serum and all four tissues compared to the placebo- and ribavirin-treated groups. Notably, in the high-dose favipiravir treatment group, there was no detectable virus found in the liver or lung tissues of one of the hamsters and a second animal had no detectable virus in the liver, kidney, or lung tissues. Due to the severity of the SFTSV infection, one hamster in the placebo group succumbed to the infection prior to the time of sacrifice and therefore could not be included in the analysis. Moreover, sufficient amounts of serum could not be obtained from the other two placebo animals because they were moribund at the time of sacrifice.

## DISCUSSION

Previous SFTSV studies in mice have demonstrated the importance of the type I IFN response in preventing severe disease in mice (10). Further, recent studies using human cells have shown that STAT2 is specifically targeted and sequestered by the NSs protein of SFTSV during infection as a mechanism for blocking the initiation of the type I IFN response program (5). Based on these findings, we hypothesized that hamsters deficient in STAT2 would be susceptible to SFTSV infection and lethal disease. In the present report, we describe a new SFTS disease model in a second species by showing



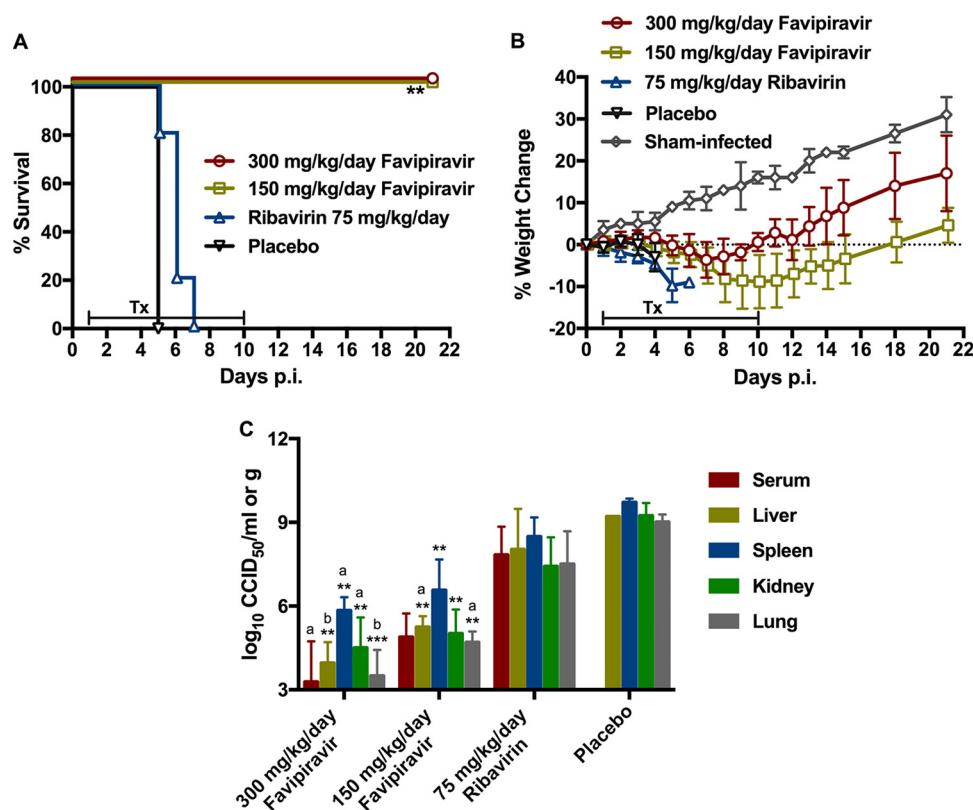


**FIG 5** Histopathologic lesions observed from the SFTSV infection in *STAT2* KO hamsters. (A to D) Representative sections of liver (arrow marking neutrophilic hepatitis) (A) and spleen (white pulp, arrow marking severe neutrophilic splenitis) (B) from an infected animal on day 4 p.i. and sections of liver (C) and spleen (white pulp) (D) from a sham-infected control animal. Bar (lower right corner of each panel), 50  $\mu$ m. Hematoxylin and eosin stain; magnification,  $\times 400$ . (E) Summary of histopathology findings represented as a heat map. Lesions were scored as follows: 0, no lesions; 1, minimal; 2, mild; 3, moderate; 4, severe.

that hamsters defective in *STAT2* function are highly susceptible to severe infection following challenge with as little as 10 PFU of SFTSV. No remarkable difference in susceptibility associated with age or gender of the *STAT2* KO hamsters was observed; however, larger cohorts to increase statistical power for such comparisons would be needed to better address this question. We also show that heterozygous littermates that possess a functional copy of the *STAT2* gene are completely refractory to infection, indicating that the lower expression levels from a single allele are sufficient to confer protection. Although we did not investigate whether the NSs protein of SFTSV interacts with and sequesters hamster STAT proteins into viral replication centers, our data suggest that this is unlikely since hamsters expressing a functional *STAT2* showed no signs of disease.

Our findings on the natural history of SFTSV infection and disease in *STAT2* KO hamsters indicate that following a 50-PFU challenge by s.c. injection, infectious viral loads reach detectable levels in the serum and most tissues in a proportion of the animals between 48 and 72 h after infection. By day 4 of the infection, significant viral loads are present in all hamsters and visible signs of illness became apparent. Consistent with a cardinal feature of the human disease (2), SFTSV infection in hamsters resulted in thrombocytopenia and the marked systemic inflammation that is common





**FIG 6** Effects of favipiravir treatment on survival outcome, percent weight loss, and virus titers in STAT2 KO hamsters challenged s.c. with SFTSV. Hamsters in each treatment group received the indicated doses of favipiravir, ribavirin, or placebo (p.o., twice daily for 10 days) beginning 1 day p.i. (A) Percent survival ( $n = 5$ /group); (B) daily percent changes and standard deviations in the mean weights of surviving animals relative to the day of virus challenge; (C) viremia and tissue viral titers on day 4 p.i. ( $n = 3$ /group). One animal in the placebo group expired prior to the time of sacrifice and was not included in the analysis. Also, sufficient serum for viremia analysis could not be obtained from the remaining 2 moribund animals in the placebo group. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  compared to animals receiving placebo. a,  $P < 0.05$ ; b,  $P < 0.01$ , compared to ribavirin-treated animals. Tx, treatment.

in hemorrhagic fever of viral etiology (14). It is possible that the observed thrombocytopenia is due to the platelet consumption often seen to occur with viral hemorrhagic fever and other coagulation disorders (15); however, we found no evidence of increased megakaryocyte production or viral cytopathic effects (CPE) in these cells. Leukopenia, a common clinical laboratory finding associated with SFTS in humans (2), was not evident in the infected hamsters.

The serum biochemistry findings were highly suggestive of liver disease. Elevations in ALP, ALT, AST, GGT, and TBIL are an indication of liver pathology (16). Specifically, AST and ALT indicate hepatocyte injury. Significant muscle damage can also cause an increase in AST, but since neutrophilic hepatitis was observed histologically, the elevated AST was due to liver dysfunction. In addition, the elevated levels of ALP, GGT, and TBIL were also the result of hepatocellular pathology as confirmed by the histopathologic lesion of neutrophilic hepatitis. Other serum biochemistry data suggestive of liver pathology were the downward trends in the levels of GLU and ALB, both products synthesized by the liver. Also, starting on day 3 p.i., the infected hamsters presented with increasing levels of CHOL, which may be secondary to cholestasis, a reduction in bile flow, suggesting hepatobiliary disease (16). The presence of cholestasis was also supported by the increases in ALP, GGT, and TBIL.

The moribund animal in the day 5 group and one of the hamsters in the day 4 group had very high BUN. The high BUN was likely due to dehydration and not renal insufficiency, since urine specific gravity was adequately concentrated in the infected animals, as in sham-infected controls and no histologic lesions were present

in kidney tissue collected from SFTSV-infected hamsters. The dramatic increase in PHOS on day 5 p.i. also suggests a decreased glomerular filtration rate secondary to dehydration (16).

Histologically, lesions were found only in spleen and liver tissues. Histopathology observed in the liver was consistent with the blood chemistry results and viral titer data, with the animals that had the most severe lesions generally having high viral loads and altered liver parameters consistent with liver dysfunction. Previous studies describing histopathology associated with SFTSV infection in mice have reported differences in tissues affected. Consistent with the study by Tani et al. using IFN- $\alpha/\beta$  receptor knockout (IFNAR $^{-/-}$ ) on a C57BL/6 mouse background and the SPL010 strain of SFTSV (9), we observed inflammatory lesions in the liver and the splenic lymphoid tissue. However, the inflammatory reaction in the hamster model was strongly neutrophilic compared to the mouse model. In contrast, we did not observe lymphoid necrosis in the lymph nodes evaluated. Interestingly, the report by Liu and colleagues using IFNAR $^{-/-}$  mice on a different background (129/Sv) and with a different strain of SFTSV (YL-1) detected SFTSV by immunohistochemistry in multiple organs, but no histologic lesions were present (10). Collectively, the results from rodent studies support the idea that type I IFN is essential but that host and virus factors, as well as challenge dose and route, can alter disease development. Our findings suggest that the STAT2 KO hamsters are succumbing from overwhelming severe acute systemic infection and inflammatory response with the likely contribution of dehydration that ultimately leads to cardiovascular failure.

Finally, we also show the STAT2 KO hamster SFTSV infection model is a suitable option to evaluate potential therapeutic treatments. The activity of favipiravir, a promising broad-spectrum antiviral with demonstrated activity against SFTSV infection in mice (9) and other related phleboviral infections in rodents (17–19), was confirmed. Of note, in contrast to the report by Tani and coworkers in which significant efficacy was observed with ribavirin at a dose of 25 mg/kg/day (9), we found the compound to be ineffective against SFTSV in STAT2 KO hamsters at a dose of 75 mg/kg/day. The difference in ribavirin efficacy may be due to a number of factors, including dosing frequency, treatment initiation time, and route of administration. Also, Tani et al. used a different strain of SFTSV (SPL010) and challenged mice with >10,000-fold more virus than the amount inoculated into hamsters. Taken together, our data support the use of the hamster SFTSV infection model to further explore the pathogenesis of SFTSV infection and for early-stage antiviral drug development efforts.

## MATERIALS AND METHODS

**Ethics statement.** All animal procedures complied with USDA guidelines and were conducted at the AAALAC-accredited Laboratory Animal Research Center at Utah State University under protocol 2383, approved by the Utah State University Institutional Animal Care and Use Committee.

**Animals.** The development of the STAT2 KO golden Syrian hamsters used has been previously described (20). Male and female 6- to 7-week-old STAT2 KO hamsters were obtained from the breeding colony at Utah State University (Logan, UT). The animals were fed autoclaved Harlan Lab Block and sterilized tap water *ad libitum* and acclimated for approximately 1 week in the biosafety level 3 (BSL-3) containment facility prior to virus challenge.

**Virus.** SFTSV strain HB29 was obtained from Robert Tesh (World Reference Center for Emerging Viruses and Arboviruses, The University of Texas Medical Branch, Galveston). The virus stock ( $5.6 \times 10^6$  PFU/ml; 1 passage in Vero E6 cells) used was derived from a clarified cell culture lysate preparation. Virus stock was diluted in sterile minimum essential medium (MEM) to achieve the desired challenge concentrations. All procedures with infectious SFTSV were conducted in BSL-3 laboratories.

**Susceptibility of STAT2 KO hamsters to SFTSV.** STAT2 KO hamsters were weighed on the day of infection and grouped by weight and gender to minimize differences across experimental groups ( $n = 4$  per virus challenge dose;  $n = 3$  for the sham-infected control group) in an initial titration study. The animals were inoculated by subcutaneous (s.c.) injection of 0.2 ml containing one of three serial dilutions of SFTSV or sham infected with MEM. The hamsters were observed for 15 days for morbidity and mortality following SFTSV challenge. Animals were weighed daily, and the percent weight changes of the infected animals relative to their starting weights were reported.

A second experiment was conducted using hamsters heterozygous for the STAT2 deletion. In this study, hamsters were challenged s.c. with 0.2 ml containing 100 PFU of SFTSV or sham infected with the

same volume of MEM. Body weight was measured daily, and the animals were observed for 12 days for morbidity and mortality associated with SFTSV infection.

**Pathogenesis and natural history of SFTSV infection in STAT2 KO hamsters.** STAT2 KO hamsters were grouped to minimize gender and weight differences and challenged with 50 PFU of SFTSV or sham infected. Predetermined groups of 3 animals per group were sacrificed on days 1 to 5 postinfection (p.i.). Animals were weighed each day prior to sacrifice, and the data were reported as the mean weights of the infected animals relative to their starting weights. Sham-infected control hamsters were sacrificed on days 1, 3, and 5 p.i. Prior to sacrifice, whole blood was collected from anesthetized animals by retro-orbital bleed directly into Sarstedt Microvette K3 EDTA-coated tubes to minimize clotting for hematologic analysis. Following euthanasia, hamsters were exsanguinated by cardiac puncture and serum was obtained for biochemistry and viral titer analyses. Urine was collected to determine urine specific gravity. Anticoagulated blood and bone marrow smears were prepared for manual white blood cell (WBC) counts and bone marrow cytologic evaluation. Tissue samples of spleen, liver, kidney, lung, brain, small intestine, heart, and lymph nodes were collected for infectious virus titer determination and histopathology.

**Viral titer determination.** Virus titers were assayed using an infectious cell culture assay as previously described (21). Briefly, tissue samples were homogenized in a fixed volume of MEM, and the homogenates and serum were serially diluted and added to triplicate wells of Vero E6 (African green monkey kidney, clone E6) cell monolayers in 96-well microtiter plates. The viral cytopathic effect (CPE) was determined 11 days after plating, and the 50% endpoints were calculated as described previously (22). The lowest limit of detection (LLD) for serum samples was  $1.67 \log_{10}$  CCID<sub>50</sub>/ml. The LLD for tissues was generally in the range of 2 to 3  $\log_{10}$  CCID<sub>50</sub>/g. In samples presenting with undetectable tissue virus titers, a value representative of the LLD was assigned for statistical purposes.

**Hematology.** Blood smears were prepared with whole blood collected in tubes coated with K3 EDTA anticoagulant. The total WBC count was estimated, a manual differential cell count was performed, and blood smears were evaluated for morphological features by a medical technologist and a board-certified veterinary clinical pathologist. WBC estimates and the percentages of immature neutrophils (Bands), neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), and eosinophils (Eos) were determined. Morphological features of toxicity were evaluated, and samples were assessed for platelet clumping.

For evaluation of erythrocyte parameters and platelets, 10% formalin was added to EDTA-anticoagulated whole-blood samples at a 1:1 ratio to inactivate virus prior to transport to the Utah Veterinary Diagnostic Lab (Logan, UT) for analysis in an automated complete blood count (CBC) instrument (Advia 120 Hematology Analyzer; Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Platelet and erythrocyte parameters, including red blood cell (RBC) counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and RBC distribution width (RDW) were determined. Several tests were performed to confirm that there were no significant differences observed in platelet and erythrocyte parameters when comparing the 10% formalin-treated to untreated EDTA-anticoagulated hamster whole blood. Because of discrepancies between the manual differential WBC numbers and the automated cell counts, we reported the manual counts since the smears were made from fresh blood samples not treated with formalin.

**Serum biochemistry.** A comprehensive 18-parameter serum biochemistry panel was performed on all serum samples to evaluate liver and kidney function as well as electrolyte and fluid balance. Tests within the panel included alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), total protein (TP), total bilirubin (TBIL), albumin (ALB), phosphate (PHOS), calcium (Ca), cholesterol (CHOL), gamma-glutamyl transferase (GGT), lipase (LIP), amylase (AMY), magnesium (Mg), triglycerides (TRIG), aspartate aminotransferase (AST), and electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>). These tests were performed using a DRI-CHEM 4000 analyzer following the manufacturer's specifications (Heska, Des Moines, IA).

**Histopathology.** Tissue samples of spleen, liver, kidney, lung, brain, small intestine, heart, and submandibular lymph nodes were preserved in 10% neutral buffered formalin and sent to the Utah Veterinary Diagnostic Laboratory (UVDL, Logan, UT) for histopathologic examination by a board-certified veterinary pathologist. In tissues where lesions were present, severity was scored on a scale of 0 to 4 with 0 indicating no lesions and 4 indicating severe lesions.

**Evaluation of favipiravir and ribavirin against SFTSV infection in hamsters.** STAT2 KO hamsters were weighed on the morning of the infection and grouped to minimize gender and weight differences across the treatment groups. Hamsters ( $n = 8$  for treatment and placebo groups,  $n = 4$  for the sham-infected group) were challenged with 50 PFU of SFTSV. Favipiravir (300 or 150 mg/kg/day) and ribavirin (75 mg/kg/day) treatments were administered twice daily for 10 days by oral gavage (p.o.) starting 1 day after challenge. Favipiravir (T-705) was obtained from the Toyama Chemical Co. (Toyama, Japan), and ribavirin was from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). Both compounds were dissolved in 0.4% carboxymethylcellulose (CMC; Sigma-Aldrich, St. Louis, MO) prior to administration. Predetermined subsets of 3 animals each were sacrificed on day 4 p.i. for analysis of serum, liver, spleen, kidney, and lung viral titers. The remaining hamsters continued to receive treatment and were observed 21 days for morbidity and mortality.

**Statistical analysis.** The Mantel-Cox log rank test was used for analysis of Kaplan-Meier survival curves. One-way analysis of variance (ANOVA) with a Tukey posttest to correct for multiple comparisons was performed to compare differences in hematologic values, blood chemistry parameters, and viral titers. All statistical evaluations were done using Prism (GraphPad Software, La Jolla, CA).

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